Expression of Cyclooxygenase-2 in Human Squamous Cell Carcinoma of the Esophagus; An Immunohistochemical Survey*

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Abstract. *Several studies indicate that the use of non-steroidal anti-inflammatory drugs (NSAIDs) may reduce the risk of esophageal cancer. The best known function of NSAIDs action is to block the enzyme cyclooxygenase, the rate limiting enzyme in the conversion of arachidonic acid to prostaglandins. In this study we investigated the expression of cyclooxygenase-2 (Cox-2) in squamous cell cancers of the esophagus and in normal esophageal squamous epithelium. Immunohistochemical detection of Cox-2 revealed strong positive staining in the well-differentiated regions of esophageal tumors, whereas histologically normal squamous epithelium stained only weakly positive. Smooth muscle cells, some stromal and inflammatory cells were also positive. Poorly differentiated areas of the esophageal tumors were negative. Our results suggest that Cox-2 is over-expressed in well-differentiated regions of squamous cell cancers of the esophagus.*

Esophageal cancer is the 9th most common cancer in the world, with areas encompassed by the "Asian esophageal cancer belt" having some of the highest incidence rates [1,2]. Areas of Shanxi Province of northern China have some of the highest mortality rates from esophageal cancer in the world, with 132 deaths each year per 100,000 people [2]. The etiologic determinants of cancer in these high risk regions remain to be identified, but it is believed that both environmental and genetic factors may be involved [3-6].

Several epidemiological studies indicate that aspirin use may reduce the risk of death from esophageal cancer [7,8]. Most recently, Farrow and colleagues [9] have reported that current users of aspirin were at reduced risk for both esophageal adenocarcinoma (odds ratio (OR), 0.37; 95% confidence interval (CI), 0.24-0.58) and squamous cell carcinoma (OR, 0.49; 95% CI, 0.28-0.87).

The best known effect of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) is to block the enzyme cyclo-oxygenase (Cox), the rate limiting enzyme in the conversion of arachidonic acid to prostaglandins [10]. Prostaglandins, such as PGE2, can promote cell proliferation, inhibit the immune response to malignant cells and may also inhibit apoptosis [11]. Two Cox genes have been cloned (Cox 1 and 2) that have approximately 60% sequence homology. Cox-1 is a housekeeping gene, while Cox-2 is an inducible, early response gene [12-16].

In-vitro experiments have shown NSAIDs to be anti-proliferative in some cell lines. Two NSAIDs, NS-398 and indomethacin, inhibited proliferation of two gastrointestinal cancer cell lines (MKN45 and CAC0-2) that over-expressed Cox-2 [17]. However, these inhibitors exerted minimal effects on proliferation of other cell lines which expressed significantly lower levels of Cox-2. In addition, aspirin and other NSAIDs have been shown to inhibit chemically induced esophageal cancer in rats [18].

Elevated levels of Cox-2 mRNA and protein, but not those of Cox-1, are found in chemically-induced rat colon carcinoma tissues [18] and in human colon carcinoma compared with normal mucosa [19]. Currently, it is unknown whether Cox-2 is expressed in esophageal tumors. If Cox-2 is expressed in esophageal tumors, then these cancers may be targets for chemoprevention by NSAIDs. Here we report an immunohistochemical survey of Cox-2 expression comparing esophageal squamous cell carcinoma specimens from a high risk Chinese population to adjacent normal esophageal

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Abbreviations: Cyclooxygenase-2; Cox-2, Non-Steroidal Anti-Inflammatory Drugs; NSAIDs.

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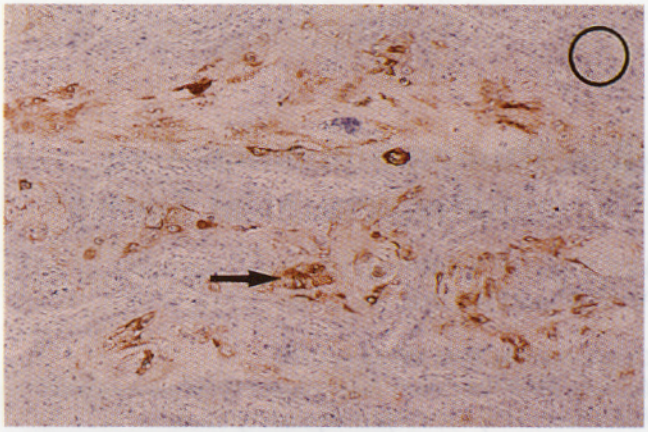

Figure 1. *Squamous cell carcinoma of the esophagus with intense (3+) brown Cox-2 staining in well differentiated regions (black arrow). The undifferentiated tumor cells are negative (open circle - blue). (Magnification 20X)*

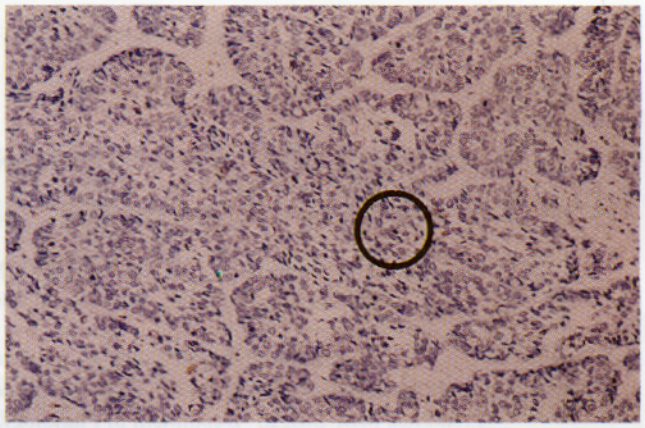

Figure 2. *Poorly differentiated squamous cell carcinoma of the esophagus, with tumor cells negative for Cox-2 (open circle - blue). (Magnification 40X).*

squamous epithelum from the same individuals in China and a sample of "low risk" individuals in the United States.

Materials and Methods

Tissue samples. Nineteen esophageal cancer specimens were obtained from patients with squamous cell carcinoma of the esophagus from a high risk population in Shanxi Province, located in northern China. This included 12 men and 7 women, with an average age of 57 years (range 39-73). Seven individuals had stage 2 disease and twelve individuals had stage 3 esophageal cancer [20]. Matched pairs of tumor and adjacent non-tumor biopsy specimens, that had been formalin fixed and paraffin embedded were histologically evaluated.

Ten normal esophageal specimens were obtained from autopsies of patients diagnosed with diseases other than esophageal cancer at the National Cancer Institute in Bethesda, Maryland (kind gift from Dr. Kleiner). The ten patients surveyed included 6 men and 4 women, with an average age of 52 years (range 14-85).

Immunohistochemistry. Formalin-fixed, paraffin-embedded tissues were cut to 4 μm sections and deparaffinized. Immunohistochemistry was performed with the immunoperoxidase technique using an anti-Cox 2 mouse monoclonal antibody from Transduction Laboratories (Lexington, KY) at a dilution of 1:400 with tyramide signal amplification (TSA-Indirect Kit, Dupont NEN Products, Boston, MA). The Vectastain Mouse Elite kit (Vector Laboratories, Burlingame, CA) was used as the secondary antibody, with diaminobenzidine (DAB) as the chromagen. Two cell lines, human colon cancer HT-29 and HCT 116 cells were used as positive and negative controls, respectively.

For each tissue specimen, the extent and intensity of staining with Cox-2 antibodies was graded as negative (-), mild (1+), moderate (2+) or intense (3+) by two pathologists. The observers assessed all tissue on the slide and staining of micro anatomical sites were also recorded.

Results

Immunohistochemical analysis revealed intense positive staining with antibodies against Cox-2 in the well differentiated tumor regions of the squamous cell carcinomas. The well differentiated regions consisted of tumor cells that were similar to normal squamous epithelial cells, with intercellular bridges and nests of keratin pearls (Figure 1). Seven of the nineteen tumors consisted predominantly of well differentiated cells that were strongly Cox-2 positive (average intensity score: 3+) (Table I). The rarer, less differentiated regions of these tumors were predominantly negative (Figure 1). Similarly, the remaining twelve moderately to poorly differentiated tumors were predominantly Cox-2 negative (Figure 2) and showed only focal, weak positive staining in their better differentiated regions (average intensity score: 2+). The histologically normal squamous esophageal epithelium from China and the USA, also expressed Cox-2 (Figure 3A and 3B), but the staining was diffuse and much weaker (average intensity score: 1.5+) compared to well differentiated regions. Immunohistochemical staining also

Table I. *Cox-2 expression in normal squamous epithelium and squamous cell carcinoma of the esophagus.*

	Number of specimens showing Cox-2 positivity (%)	Average intensity score
Normal squamous epithelium (N=29)	26 (90%)	1.5+[a]
Well differentiated tumors (N=7)	7 (100%)	3+[b]
Moderately to poorly differentiated tumors (N= 12)	7 (58%)	2+[b]
Stroma (N=29)	23 (79%)	2+[c]
Smooth muscle (N=29)	27 (93%)	3+[d]

[a] Staining uniform and diffuse.
[b] Average intensity in well differentiated regions. Undifferentiated regions are negative.
[c] Staining in focal regions.
[d] Average intensity in all smooth muscle cells.

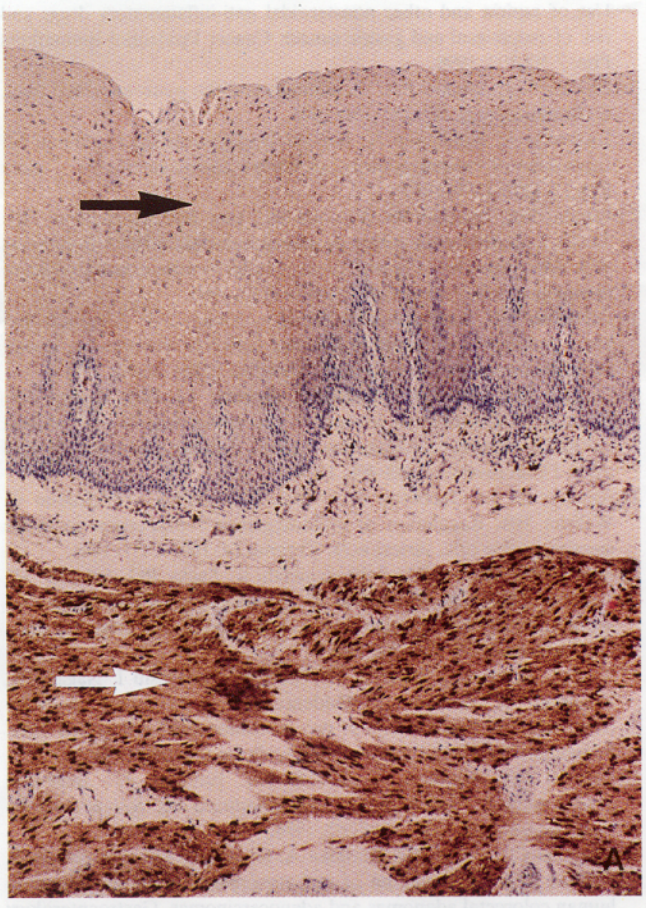

Figure 3A. *Histologically normal squamous esophageal epithelium from a Chinese patient with diffuse mild staining (1.5+) for Cox-2 (black arrow). The intensely (3+) stained brown cells at the bottom are Cox-2 positive smooth muscle cells (white arrow). (Magnification 10X)*

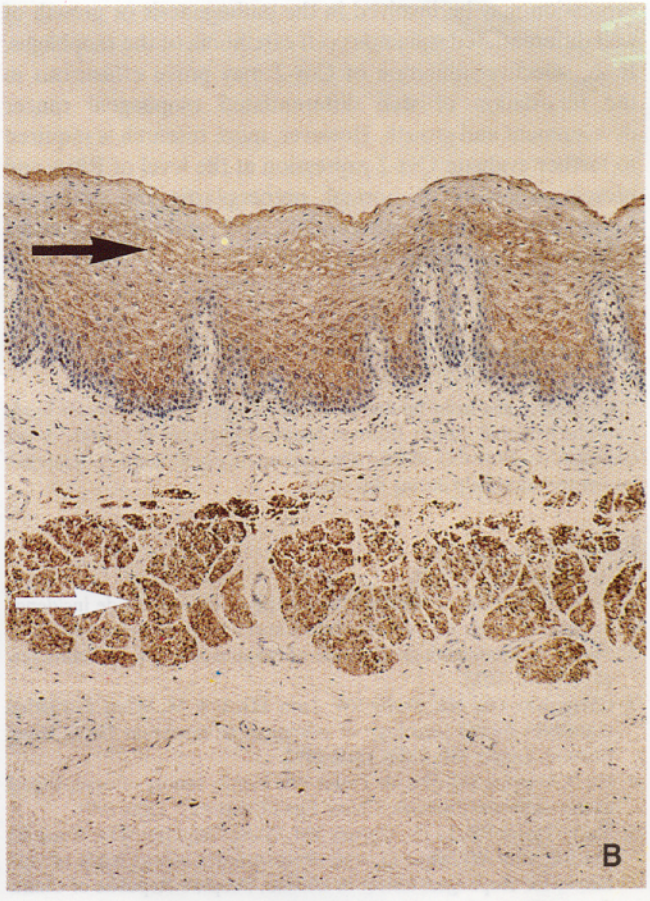

Figure 3B. *Histologically normal squamous esophageal epithelium from a US patient with diffuse mild staining (1.5+) for Cox-2 (black arrow). The brown stained (2+) cells below the epithelium are Cox-2 positive smooth muscle cells (white arrow). (Magnification 10X)*

demonstrated intense positivity in smooth muscle cells (Figures 3A and 3B), scattered inflammatory cells and some foci of stromal cells.

Discussion

Using immunohistochemical techniques, we found stronger staining for Cox-2 protein in well differentiated regions of human squamous esophageal carcinomas than in poorly differentiated areas of these tumors or in normal esophageal squamous epithelium. To our knowledge, this is the first report of elevated Cox-2 expression in human esophageal squamous tumors. Increased Cox-2 expression has been previously identified in human colorectal and gastric adenocarcinomas [11]. Thus, epithelial cancers throughout the gastrointestinal tract may over-express Cox-2. In our esophageal specimens we also observed diffuse weak staining of histologically normal squamous epithelial cells, positive staining of stromal CT cells and intense positive staining of some stromal inflammatory cells and smooth muscle cells. The immunolocalization of Cox-2 in smooth muscle cells is similar to that reported by Hwang and colleagues [21]. Poorly differentiated portions of the squamous esophageal cancers did not stain for Cox-2.

Our results, although qualitative in nature, suggest that expression of Cox-2 is elevated in well differentiated regions of squamous cell carcinomas of the esophagus. Cox-2 over-expression may contribute to the pathogenesis or growth of these cancers by enhancing synthesis of prostaglandin E2, increasing cell proliferation, immune-suppression and inhibition of apoptosis [11]. Alternatively, Cox-2 over-expression in these tumors may merely be a marker of squamous differentiation in an abnormal setting, without significant effect on the neoplastic process.

The reason for Cox-2 expression in histologically normal squamous epithelium of our esophageal sections is not known, but it seems represent constitutive expression because it was seen in 90% of the specimens tested in both the "high risk" Chinese and the "low risk" US patients. To our knowledge, this is the first report of Cox-2 immuno- staining in normal esophageal mucosa.

Our findings raise the possibility that Cox-2 over-

expression may be involved in the pathogenesis or growth of well differentiated squamous cell carcinoma of the esophagus. If so, selective inhibition of Cox-2 may prove efficacious in the retardation of well differentiated esophageal cancer development and growth. However, more research is required to further evaluate Cox-2 expression at the level of RNA and protein expression in normal, preneoplastic and cancerous lesions and to confirm differential Cox-2 expression in tumors at different stages of differentiation.

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